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Description of a New Species of the Genus *Brachytarsophrys* Tian and Hu, 1983 (Amphibia: Anura: Megophryidae) from Southern China Based on Molecular and Morphological Data

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Abstract A new species, *Brachytarsophrys popei* sp. nov., is described based on a series of specimens collected from Mount Jinggang, Jiangxi Province, Taoyuandong Nature Reserve, Hunan Province and Nanling Nature Reserve, Guangdong Province, China. The new species can be easily distinguished from other known congeners by morphology, morphometrics and molecular data of the mitochondrial 16S rRNA gene. It is characterized by its relatively small size with 86.2 mm in snout-vent length in adult female and 70.7 mm–83.5 mm in males; vomerine teeth bearing on two markedly elevated ridges, which projecting behind far beyond the posterior level of the choanae, widely separated by a distance nearly 1.5 times length of one; margin of tongue deeply notched behind; toes about one-third to two-thirds webbed in males, at most one-third webbed in female; the webs extending as a wide fringes along either side of toes; upper eyelid with tubercles, one of which is enlarged and becoming a remarkably prominent, bluntly conical light-yellow horn; black tiny nuptial spines on the dorsal surface of the first finger and second finger base, single vocal sac in males; gravid females bear pure yellowish oocytes; tadpoles with a transverse white stripe on ventral surface and two longitudinal white stripes along the sides of body. The new species represents the fifth known *Brachytarsophrys* species.

Keywords Megophryidae, *Brachytarsophrys popei* sp. nov., morphology, mitochondrial DNA, taxonomy

1. Introduction

The genus of *Brachytarsophrys* was originally established by Tian and Hu (1983), with the *Megophrys carinense* (=*Brachytarsophrys carinense*) assigned as the type species of the genus. Dubois (1987 "1986") considered it as a subgenus of *Megophrys*. Currently, the monophyly and validity of the genus *Brachytarsophrys* is widely accepted by morphological and phylogenetic studies (Xie and Wang, 2000; Frost *et al*., 2006; Fei *et al*., 2009; Pyron and Wiens, 2011). In their review on the populations of *Brachytarsophrys* from China, Rao and Yang (1997) described a new species as *B. platyparietus* from northern Yunnan, further noted that *B. carinense* is only distributed in tropical forests in Myanmar and Thailand whereas the widely distributed populations of *Brachytarsophrys* that used to be recognized as *B. carinense* in southern and southwestern China is *B. platyparietus*, and *B. feae* only occurs in Yunnan-Myanmar border. Later, another new taxon of the genus, *B. chuannanensis*, was described by Fei, Ye and Huang (Fei and Ye, 2001) from southern Sichuan Province, China. Fei *et al*. (2009) argued that *B. platyparietus* should be listed a synonym of *B. carinense*, and also noted that they would rather treat the widely distributed populations of *Brachytarsophrys* from Southern China as *B. carinense*, before getting the topotype specimens of *B. carinense* for comparison. Thus, the validity of *B. platyparietus* still remains uncertain. So, excluding *B. platyparietus*, there are currently only four species of the genus *Brachytarsophrys* recognized, i.e., *B. carinense* (Boulenger, 1889) widely distributed

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from southern Myanmar, northern Thailand and southern China; *B. chuannanensis* Fei, Ye, and Huang, 2001 only recorded from southern Sichuan of China; *B. feae* (Boulenger, 1887) distributed from northern Myanmar, northern Thailand, northern Vietnam and southern Yunnan of China; *B. intermedia* (Smith, 1921) only occurred in southern Vietnam and Laos (Fei *et al*., 2009; Frost, 2014).

In this paper, we re-evaluate the taxonomic status of the populations of *Brachytarsophrys* from Mount Jinggang of Jiangxi, Taoyuandong Nature Reserve of Hunan and Nanling Nature Reserve of Guangdong, southern China based on morphological and molecular data of the mitochondrial 16S rRNA gene. Our results suggest that the populations of *Brachytarsophrys* from southern China belong to the same species which can be distinguished from *B. carinense* and other congeners by discrete morphological differences and genetic divergence. Therefore, we describe these populations from southern China as a new species.

2. Material and Methods

2.1 Taxon sampling Samples used for molecular analyses including the specimens SYS a001485-1, 1876–1878 from Mount Jinggang, Jinggangshan City, Jiangxi Province, the specimens SYS a001864–1866 from Taoyuandong Nature Reserve (NR), Yanling County, Hunan Province, the specimen SYS a000589 from the Nanling Nature Reserve (NR), Ruyuan County, Guangdong Province for an undescribed *Brachytarsophrys* species; the specimens SYS a001770 and 1771 from Heping, Zhenyuan County, Yunnan Province for *B. feae*; the sample from the Zihuai, Hejiang County, Sichuan Province for *B. chuannanensis* (Figure 1). All specimens were fixed in 1% buffered formalin after preserving muscle tissue in 95% ethanol, and later transferred to 70% ethanol. The sample of *B. chuannanensis* deposited at the Chengdu Institute of Biology (CIB), the Chinese Academy of Sciences (CAS), and other specimens deposited at the Museum of Biology, Sun Yat-sen University (SYS).

2.2 Extraction, PCR and sequencing DNA was extracted from muscle tissue using a standard phenol-chloroform extraction protocol (Sambrook *et al*. 1989). The mitochondrial 16S rRNA gene from all taxon samples was sequenced. Fragments of the

Figure 1 Collecting localities in southern and southwestern China: I: Mount Jinggang, Jinggangshan City, Jiangxi Province, and Taoyuandong Nature reserve, Yanling County, Hunan Province, specimens collected here refer to undescribed *Brachytarsophrys* sp.; II: Nanling Nature Reserve, Ruyuan County, Guangdong Province, specimens collected here refer to *B.* sp.; III: Heping Town, Zhenyuan County, Yunnan Province, specimens collected here refer to *B. feae*; IV: Hejiang County, Sichuan Province, specimen collected here refer to *B. chuannanensis*.

genes were amplified using primer pairs L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3'). PCR amplifications were performed in a reaction volume of 30 μl containing 75 ng of template DNA, 20.7 μl of ddH_2O, 3 μl of 10*PCR Buffer, 1.2 μl of 25 mM $Mgcl_2$, 2 μl of 2.5 mM dNTP, 0.7 μl of 10 μM L3975, 0.7 μl of 10 μM H4551 and 0.2 μl Taq™ (Takara, Dalian, China) under the following cycling conditions: an initial denaturation step at 95 °C for 4 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, followed by a cycle extension at 72 °C for 1 min, and a final extension step of 72 °C for 7 min. PCR products were purified with spin columns. The purified PCR products were sequenced with both forward and reverse primers using the ABI Big-Dye Terminator v 3.1 Cycle Sequencing Kit according to the guidelines of the manufacturer. The products were sequenced on an ABI Prism 3730 automated DNA sequences in Shanghai Majorbio Bio-pharm Technology Co., Ltd. All sequences have been deposited in GenBank (Table 1).

2.3 Phylogenetic analysis Sequences of *Brachytarsophrys carinense* and *B. intermedia* from GenBank. *Xenophrys cheni* and *X. lini* from Taoyuandong NR, Hunan and Mt. Jinggang, Jiangxi were used as the out-group. Data of all voucher specimens of above species are presented in Table 1. Alignments were first conducted using Clustal X 2.0 (Thompson *et al.*, 1997) in MEGA 6.05 (Tamura *et al.*, 2011), with default parameters and the alignment being checked and manually revised, if necessary. The GTR model (Posada and Crandall, 2001), assuming a gamma-shaped distribution across sites (Felsenstein, 2004), was selected as the best-fitting nucleotide substitution model using MEGA 6.05. Sequence data were analyzed using maximum likelihood (ML) implemented in MEGA 6.05, and Bayesian inference (BI) using MrBayes 3.12 (Ronquist and Huelsenbeck, 2003). The phylogenetic tree was constructed using ML and BI methods. For ML analysis, the bootstrap consensus tree inferred from 1000 replicates was used to estimate nodal supports of inferred relationships on phylogenetic tree. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. For BI analysis, two independent runs with four Markov Chain Monte Carlo simulations were performed for one million

Table 1 Localities and voucher data for all specimens used in this study.

ID	species	Locality	Specimen voucher No.	GenBank Accession No.
1	*Brachytarsophrys* sp.	China: Naling NR, Guangdong	SYS a000589	KM504251
2	*Tadpole* of *B.* sp.	China: Mount Jinggang, Jiangxi	SYS a001485-1	KM504252
3	*B.* sp.	China: Mount Jinggang, Jiangxi	SYS a001876	KM504253
4	*B.* sp.	China: Mount Jinggang, Jiangxi	SYS a001877	KM504254
5	*B.* sp.	China: Mount Jinggang, Jiangxi	SYS a001878	KM504255
6	*B.* sp.	China: Taoyuandong NR, Hunan	SYS a001864	KM504256
7	*B.* sp.	China: Taoyuandong NR, Hunan	SYS a001865	KM504257
8	*B.* sp.	China: Taoyuandong NR , Hunan	SYS a001866	KM504258
9	*B. feae*	China: Heping, Zhenyuan Co., Yunnan	SYS a001770	KM504259
10	*B. feae*	China: Heping, Zhenyuan Co., Yunnan	SYS a001771	KM504260
11	*B. carinense*	Myanmar: –	–	JN848360
12	*B. intermedia*	Vietnam: U Bo, Phong Nha-Ke Bang NP	ZFMK 87596	HQ588950
13	*B. intermedia*	Vietnam: U Bo, Phong Nha-Ke Bang NP	ZFMK 86980	HQ588949
14	*B. chuannanensis*	China: Zihuai, Hejiang Co., Sichuan	CIB20050081	KM504261
15	*Xenophrys cheni*	China: Taoyuandong NR, Hunan	SYS a002124	KJ560397
16	*X. cheni*	China: Taoyuandong NR, Hunan	SYS a002142	KJ560398
17	*X. lini*	China: Mt. Jinggang, Jiangxi	SYS a002382	KJ560414

iterations and sampled every 100th step. The first 25% of samples were discarded as burn-in. Convergence of the Markov Chain Monte Carlo simulations was assessed using Tracer *v*.1.4 (http://tree.bio.ed.ac.uk/software/tracer/). Apart from phylogenetic tree-based methods, we also calculated pairwise sequence divergence based on uncorrected *p*-distance implemented in MEGA 6.05.

2.4 Morphometrics Measurements were made with digital calipers to the nearest 0.1 mm. Abbreviations used are SVL = snout–vent length; HDL = head length from tip of snout to the articulation of the jaw; HDW = head width, between left and right articulations of the quadratojugal and maxilla; SNT = snout length, from tip of snout to the anterior corner of the eye; ED = eye diameter, from the anterior corner of the eye to posterior corner of the eye; IND = internasal distance; IOD = interorbital distance; HND = hand length, from distal end of radioulna to tip of distal phalanx of III; RAD = radioulna length; FTL = foot length, from distal end of tibia to tip of distal phalanx of III; TIB = tibial length; TaL = tail length in tadpole, was measured from the tip of the tail fin to the vent.

All studied specimens and materials for DNA analysis are deposited in The Museum of Biology, Sun Yat-sen University (SYS), Guangzhou, Guangdong Province, China.

3. Results

3.1 Molecular analysis For the 406 bp 16S rRNA gene, a total of eleven sequences were obtained from individuals in our study and six sequences were downloaded from GenBank (Accession Nos. see Table 1). The two phylogenetic approaches resulted in the virtually identical topology indicating that the monophyly of eight individuals from Mount Jinggang, Taoyuandong NR and Nanling NR were strongly supported (99%, and 0.99 for ML bootstrap proportions and Bayesian posterior probability, respectively; Figure 2). The smallest pairwise genetic distance was only 0.031 (uncorrected p-distance) between *B. feae* and *B. chuannaensis*, whereas the smallest pairwise genetic distance between the *B.* sp. and other four known species of genus *Brachytarsophrys* was 0.038 (see Table 2). Combined with further evidence from morphology, our results indicate that the individuals from the Mount Jinggang, Taoyuandong Nature Reserve and Nanling Nature Reserve represent an undescribed species of the genus *Brachytarsophrys*. Herein, we described it as a new species, *B. popei* sp. nov.

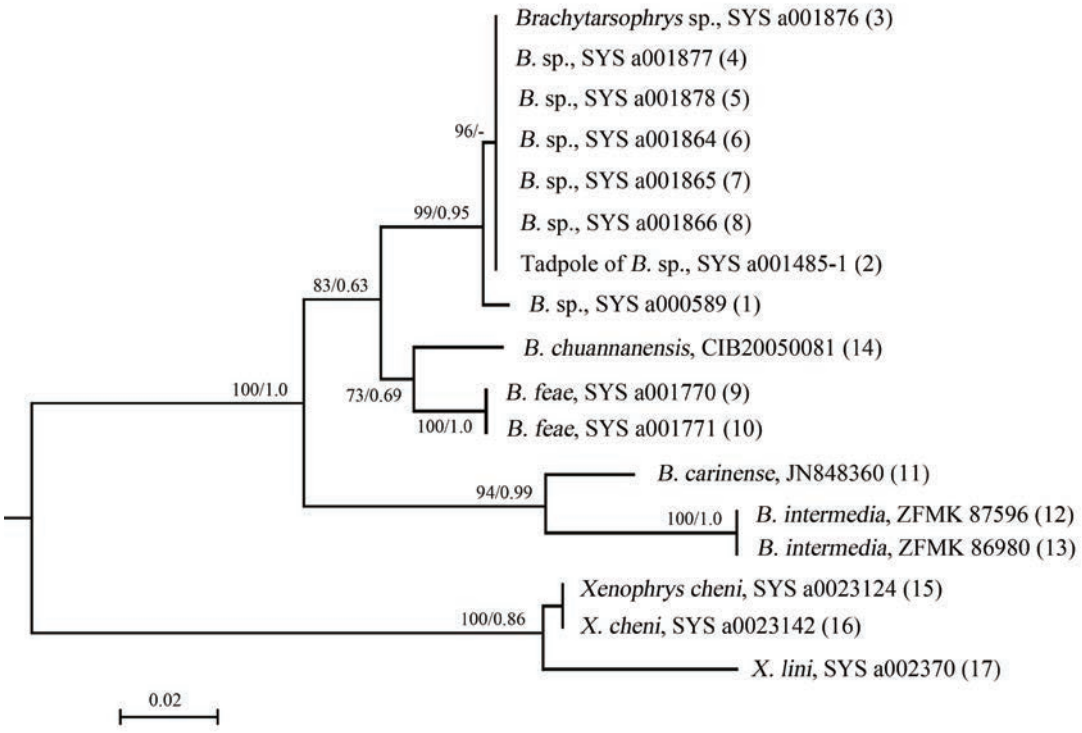

Figure 2 Bayesian inference tree derived from partial DNA sequences of the mitochondrial 16SrRNA gene. Numbers above branches are bootstrap support for maximum parsimony (1000 replicates) analyses (> 70 retained) and numbers below branches indicate Bayesian posterior probabilities (> 90% retained).

3.2 Taxonomic Account *Brachytarsophrys popei* Wang, Yang and Zhao sp. nov.

Holotype: Adult male, SYS a001867 (Figure 3 A and C; Figure 4 A and D), collected by Jian Zhao, Jianhuan Yang and Runlin Li on 17 July 2012, from Taoyuandong Nature Reserve (26°30'8.79" N, 114°03'38.27" E; 1045 m a.s.l.), Yanling County, Hunan Province, China.

Paratypes: 13 specimens (one adult female, 12 adult males): SYS a001875 (Figure 3 B and D; Figure 4 E), adult female; SYS a001874 and 1876–1878, adult males, collected by Jian Zhao, Jianhuan Yang and Runlin Li on 20 July 2012, from Mount Jinggang (26°29'51.85" N, 114°04'50.68" E; 923 m–1270 m a.s.l.), Jinggangshan City, Jiangxi Province, China. SYS a001864–1866, adult males, collected from the same locality as holotype at 874 m–1050 m a.s.l. by Jian Zhao, Jianhuan Yang and Runlin Li on 17 July 2012. SYS a000583–0585 and 0588–0589, adult males, collected by Jianhuan Yang and Runlin Li on 13 August 2009, from Nanling Nature Reserve (24°56'14.19" N, 113°0'13.12" E; 1089 m–1304 m a.s.l.), Ruyuan County, Guangdong Province, China.

Other examined materials: Fourteen tadpoles, collected from Bamianshan and Jingzhushan of Mount Jinggang at 800 m–1270 m a.s.l. by Jian Zhao and Runlin Li; six of them on 7 April 2011, eight on 6 December 2011 (Figure 3 E and F).

Diagnosis: *Brachytarsophrys popei* sp. nov. is characterized by the combination of following characters: (1) A relatively small-sized Short-legged Toad, SVL 86.2 mm in adult female, 70.7 mm–83.5 mm in adult males; (2) head enormous, and extremely depressed, nearly 1.2 times as broad as long, nearly one-half as broad as the SVL; (3) canthus rostralis not developed; (4) tympanum hidden; (5) vomerine ridges elevated, narrow and long, projecting behind far beyond the posterior level of the choanae, widely separated by a distance nearly 1.5 times length of one; (6) tongue pyriform, deeply notched behind; (7) the heels not meeting; (8) tibio-tarsal articulation reaching to the commissure of the jaw; (9) relative finger length II < I < IV < III; (10) toes about one-third to two-thirds webbed in males, at most one-third webbed in female; (11) the fringes significantly wide along either side of toes in males, slightly wide in female; (12) upper eyelid with tubercles, one of which is enlarged and becoming a remarkably prominent, bluntly conical, light-yellow horn; (13) tiny, darker brown nuptial spines densely covering

Table 2 Genetic divergence of the *Brachytarsophrys* and *Xenophrys* species studied based on uncorrected *p*-distance in a 16S rDNA fragment.

Species (ID No.)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
Brachytarsophrys sp. (1)	-															
Tadpole of *B.* sp. (2)	0.007	-														
B. sp. (3)	0.007	0.000	-													
B. sp. (4)	0.007	0.000	0.000	-												
B. sp. (5)	0.007	0.000	0.000	0.000	-											
B. sp. (6)	0.007	0.000	0.000	0.000	0.000	-										
B. sp. (7)	0.007	0.000	0.000	0.000	0.000	0.000	-									
B. sp. (8)	0.007	0.000	0.000	0.000	0.000	0.000	0.000	-								
B. feae (9)	0.041	0.038	0.038	0.038	0.038	0.038	0.038	0.038	-							
B. feae (10)	0.041	0.038	0.038	0.038	0.038	0.038	0.038	0.038	0.000	-						
B. carinense (11)	0.077	0.080	0.080	0.080	0.080	0.080	0.080	0.080	0.077	0.077	-					
B. intermedia (12)	0.090	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.087	0.087	0.046	-				
B. intermedia (13)	0.090	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.087	0.087	0.046	0.000	-			
B. chuannanensis (14)	0.046	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.031	0.031	0.080	0.082	0.082	-		
Xenophrys cheni (15)	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.106	0.106	0.118	0.118	0.118	0.104	-	
X. cheni (16)	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.106	0.106	0.118	0.118	0.118	0.104	0.000	-
X. lini (17)	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.109	0.109	0.136	0.127	0.127	0.112	0.038	0.038

Figure 3 General aspect of *B. popei* sp. nov. A: Dorsolateral view of the adult male holotype SYS a001867 in life. B: Dorsolateral view of the adult female paratype SYS a001875 in life. C: Ventral view of the holotype SYS a001867 in life. D: Ventral view of the paratype SYS a001875 in life. E: Lateral view of tadpole at Stage 27 in preservative. F: Ventral views of the tadpole at Stage 27 in preservative. Photo by Yingyong WANG and Jianhuan YANG.

the dorsal surface of the first finger and second finger in adult males; (14) single vocal sac in males; (15) tadpoles with a transverse white stripe, posteriorly white speckles on ventral surface and two longitudinal white stripes along the sides of body.

Description of Holotype: Adult male; the body stout,

Figure 4 Morphological differences between of *B. popei* sp. nov., *B. chuannanensis* and *B. feae*. A, B and C: Dorsal view of head in holotype SYS a001867 of *B. popei* sp. nov., holotype CIB98A0045 of *B. chuannanensis* and SYS a001771 of *B. feae*, respectively. D and E: Soles of the feet in male holotype SYS a001867 and female paratype SYS a001875 of *B. popei* sp. nov., respectively; F: Sole of the foot in male holotype CIB98A0045 of *B. chuannanensis*. G: Soles of the feet in male SYS a001770 of *B. feae*. H: Ventral view of tadpole at Stage 44 of *B. feae* in preservative. Photo by Yingyong WANG.

SVL 72.9 mm; head enormous and extremely depressed, nearly 1.2 times as broad as long, and nearly one-half as broad as the SVL; snout short, rounded in dorsal view, sloping forward to mouth in profile, slightly protruding beyond margin of lower jaw; canthus rostralis indistinct; loreal region very oblique, slightly concave; nostril closing to the tip of snout; internasal rigion slightly concave; interorbital region flatted; interorbital distance significantly wider than an upper eyelid, 1.63 times as broad as internasal distance; occipital region significantly uplifted, forming two circular bulges and a longitudinal concave groove along the middle line across occiput; a distinct transverse groove defines the head behind; eye large, eye diameter 25% of head length; pupil vertical; temporal region significantly oblique; tympanum completely hidden; choanae large, ovoid, partly concealed by the maxillary shelves; two vomerine ridges markedly elevated, extending behind far beyond the posterior level of the choanae, widely separated by a distance nearly 1.5 times length of one, bearing about 20 prominent teeth; tongue pyriform, deeply nicked behind.

Forelimbs short and moderately robust; radioulna length 22% SVL, hands without web, moderately longer, 26% of SVL; fingers rather short, relative finger length II < I < IV < III; tips of digits round, feebly dilated; no lateral fringes; metacarpal tubercle two, inner one significantly enlarged, outer one slightly enlarged. Hindlimbs relatively short and robust; tibio-tarsal articulation reaching to the commissure of the jaw, when leg stretched along the side of the body; the heels separated, when the flexed legs are held at right angles to the body axis; tibia length 40% of SVL; foot length 60% of SVL; relative toe lengths I < II < V < III < IV; tips of toes round, moderately dilated; toes with markedly developed webs, first and second toes about two-thirds webbed, third and fifth nearly one-half webbed, fourth up to one-third webbed; the web extending as a wide fringe along either side, nearly two-third as broad as distal phalanx of toe; no subarticular tubercle; tarsal fold absent; inner metatarsal tubercle ovoid, longer than the first toe; outer metatarsal tubercle absent.

Skin on all upper surfaces smooth with scattered several conical tubercles on flank of trunk and dorsum of body and limbs; upper eyelid with tubercles, one of which is enlarged and becoming a rather short, remarkably prominent, bluntly conical horn; supratympanic fold narrow and distinct, curving posteroventrally from posterior corner of eye to a level above insertion of arm; ventral skin of body and limbs smooth with granules; pectoral gland distinct, closer to axilla than to mid-ventral line; rear of thigh with a small femoral gland, around which densely arranged granules forming a granular patch; tiny nuptial spines densely covering the dorsal surface of the first finger and second finger; cloacal opening unmodified, directed posteriorly, at upper level of thighs.

Measurements of holotype (in mm): SVL 72.9, HDL 30.8, HDW 36.3, SNT 8.9, IND 7.5, IOD 12.2, ED 7.8, HND 19.1, RAD 6.1, FTL 43.9, TIB 28.9.

Coloration of holotype in life: Dorsum brown, tinged with dark blotches and streaks; of which, a yellowish-edged dark brown streak between the two eyes; posteriorly a indistinct dark brown reverse V-shaped marking between two shoulders, apex of the V-shaped marking on the occiput; tubercles black on back of body, light brown or brown tinged with black on sides of body; upper lip light brown; tympanic region brown, mottled with dark brown markings; forearm with a wide, dark brown oblique band; dorsal digits with dark brown transverse bands; ventral surface brown-black with small white granular spots; pectoral gland yellowish; femoral gland white; several yellowish spots on two sides of belly; lower surface of digits pale blue-grey; webs, palms and soles blue-grey; tip of digits, two metacarpal tubercle and inner metatarsal tubercle grey-white; nuptial spines darker brown; the tubercles at upper eyelid light colored, horn yellowish; pupils black; iris brownish.

Coloration of holotype in preservative: Dorsal surface of head pale brown, upper lip cream, remainder of body a dark reddish-brown; yellowish spots, pectoral gland and tubercles fades to grey-white.

Description of tadpole: Body slender, oval, flattened above; tail depth slightly larger than body depth; dorsal fin arising just before origin of tail, maximum depth near mid-length, tapering gradually to narrow, pointed tip; tail 2.2–2.8 times as long as body length of tadpoles in 26th stages; tail 2.3–2.4 times as long as body length in 27th stages, 2.0–2.1 times as long as body length in 29th stages; eyes large, lateral; nostril dorsolateral, slightly closer to eye than toumbelliform oral disk; internasal wider than interorbital; spiracle on left side of the body, closer to eye than to end of body; anal tube extends backward above ventral fin, opening medial; oral disk terminal, lips expanded and directed upwardly, rim raised (see Table 3 and Figure 3 E and F).

Color of tadpole in preservative: Body brown to dark brown; the presence of unique color patterns on lower surface of body: two short, longitudinal white stripes bordered with blackish-brown on sides of ventral surface of head; posteriorly a transversal white stripe bordered

Table 3 Measurements (minimum–maximum, mean; in mm) of tadpoles of *B. popei* sp. nov.

Stage	26th (n = 9)	27th (n = 3)	29th (n = 2)
SVL	7.0–9.1, 8.1	7.5–9.4, 8.1	10.0–10.3, 10.2
Tal	15.7–21.9, 20.2	17.2–21.9, 19.1	19.9–21.7, 20.8
TaL/SVL	2.2–2.8, 2.5	2.3–2.4, 2.3	2.0–2.1, 2.1

with blackish-brown left reaching to the spiracle; two longitudinal white stripes along the sides of body, which beginning from end-points of the white transversal stripe extended backward to near the anal tube; belly mottled with dense white speckles between two longitudinal stripes; tail with three dark longitudinal stripes, one at base of the dorsal fin, one at base of the ventral fin, one on either side of tail along its middle line (Figure 4 E and F).

Variation: Measurements and body proportions of type series are given in Table 4.

All 12 adult male paratypes are very similar to the holotype in morphology and color pattern. The female Paratype SYS a001875 differs from males by its body reddish brown, upper lip swollen and lighter colored; webs and fringes on toes significantly smaller (toes at most one-third webbed); occipital region flattened, not uplifted; transverse groove slightly distinctly defines the head behind; femoral gland indistinct.

Male secondary sexual characteristics: The presence of single vocal sac; rear of thigh with a small femoral gland; indistinct nuptial pad on the dorsal surface of the first finger and second finger base, with dense tiny black nuptial spines in all adult male specimens.

Etymology: The specific name "*popei*" is in honour of the noted American herpetologist Clifford H. Pope (1899–1974), in recognition of his efforts on biodiversity surveys and research of amphibians and reptiles in Southeast China. We propose the standard name "Pope's Short-legged Toad", chinese name "Po Shi Duan Tui Chan".

Distribution: Currently, *B. popei* sp. nov. is distributed in the middle section of Luoxiao Mountains and Nanling Mountains in southern China, including three type localities: the Mount Jinggang, Jiangxi Province, the adjacent Taoyuangdong NR, Hunan Province, and the Nanling NR, Guangdong Province, China. In addition, the Yizhang County, Hunan Province bordered with the Nanling Nature Reserve, thus, population as *B. carinense* from Yizhang County of Hunan should be the *B. popei* sp. nov.

3.3 Comparisons Comparative data for *Brachytarsophrys popei* sp. nov. and known four species of *Brachytarsophrys* were obtained from the literature (Boulenger, 1889, 1890, 1908; Simith 1921; Taylor, 1962; Fei and Ye, 2001; Fei *et al*., 2009). The specimens of *B. carinense* which was described as *B. platyparietus* by Rao and Yang (1997), *B. Chuannanensi*s, and *B. feae* examined first hand are listed in Appendix 1.

B. popei sp. nov**.** can be steadily distinguished from all known four congeners by its body size relatively small, measuring 70.7 mm–83.5 mm in SVL in adult males, 86.2 mm in adult female *vs.* body size large, 91.6 mm–123 mm in male and 124 mm–168 mm in females in *B. carinense* (Boulenger, 1889, 1908; Taylor, 1962; Fei *et al.* 2009); 91.4 mm–109.4 mm in adult males in *B. chuannanensis* (Fei and Ye, 2001); 78 mm–101.8 mm in adult males, 91 mm–135 mm in adult females, maximum SVL 139.7 mm

Table 4 Measurements (minimum–maximum, mean ± SD; in mm) of the type series of *B. popei* sp. nov.. See Materials and Methods for abbreviations.

	males (n = 13)	females (n = 1)
SVL	70.7–83.5 (76.9 ± 3.49)	86.2
HDL	29.8–34.2 (32.2 ± 1.29)	36
HDW	36.0–40.8 (39.1 ± 1.62)	42.5
SNT	8.5–10.3 (9.6 ± 0.54)	10.6
IND	7.0–8.7 (7.8 ± 0.44)	8.2
IOD	11.1–14.0 (12.0 ± 1.16)	13.9
ED	7.8–10.6 (9.3 ± 1.04)	9.8
HND	18.6–21.6 (19.8 ± 0.95)	20.5
RAD	15.6–17.9 (16.9 ± 0.82)	17.3
TIB	28.0–33.5 (30.8 ± 1.63)	31.4
FTL	42.0–49.7 (46.1 ± 2.25)	49.5
HDL/SVL	0.40–0.43 (0.42 ± 0.01)	0.42
HDW/SVL	0.47–0.53 (0.51 ± 0.02	0.49
HDW/HDL	1.18–1.26 (1.21 ± 0.03)	1.18
SNT/HDL	0.28–0.31 (0.30 ± 0.01)	0.29
SNT/SVL	0.12–0.13 (0.13 ± 0.00)	0.12
IND/HDW	0.18–0.21 (0.20 ± 0.01)	0.19
IOD/HDW	0.29–0.35 (0.31 ± 0.02)	0.33
ED/HDL	0.25–0.33 (0.29 ± 0.02)	0.27
ED/SVL	0.11–0.14 (0.12 ± 0.01)	0.11
HND/SVL	0.24–0.28 (0.26 ± 0.01)	0.24
RAD/SVL	0.20–0.24 (0.22 ± 0.01)	0.2
TIB/SVL	0.38–0.43 (0.40 ± 0.01)	0.36
FTL/SVL	0.57–0.63 (0.60 ± 0.02)	0.57

(= 5.5 inches) in *B. feae* (Boulenger, 1890, 1908; Taylor, 1962; Fei *et al.*, 2009); 86 mm–103 mm in male type specimens, 92 mm in adult female type specimen in *B. intermedia* (Smith, 1921); tongue deeply notched behind *vs.* indistinctly or feebly notched in *B. carinense*, feebly notched in *B. chuannanensis*, *B. feae* and *B. intermedia*; toes one-third to two-thirds webbed in males (Figure 4 D), at most one-third webbed in female (Figure 4 E) *vs.* with a mere rudiment of web at the base in *B. chuannanensis* (Figure 4 F) and *B. feae* (Figure 4 G); the lateral fringe markedly enlarged along either side of each toe, two-third as broad as distal phalanx of toe in males (Figure 4 D), nearly one-third as broad as distal phalanx of toe in female (Figure 4 E) *vs.* the lateral fringe distinct, rather narrow, at most one-tenth as broad as distal phalanx of toe in *B. chuannanensis* (Figure 4 F) and *B. feae* (Figure 4 G); having two longitudinal white stripes along the sides of body, belly mottled with dense white speckles between two longitudinal stripes in tadpoles (Figure 3 E and F) *vs.* absent the same white stripes along the sides of body in *B. chuannanensis* and *B. carinense*, belly with irregular transversal pale stripes and lacking speckles in *B feae* (Figure 4 H); its vomerine ridges long, extending behind far beyond the posterior level of the choanae, separated by a distance nearly 1.5 times length of one *vs.* vomerine ridges small, extending behind the posterior level of choanae, separated by a distance equivalent to four times length of one in *B carinense*, vomerine ridges small and widely separated just behind level of choanae in *B. feae* and *B. intermedia*; canthus rostralis indistinct *vs.* well-marked in *B. carinense*, distinct in *B. chuannanensis*, *B. feae* and *B. intermedia*.

Furthermore, new species differs from *B. carinense* by its skin above without elevated ridges (Figure 3 A and B) *vs.* generally with paired dermal ridges on back from head to shoulders, a pair of elevated dorsolateral small ridges and a pair of irregular ridges above groin in latter. From *B. feae* by its skin above smooth *vs.* skin with bony deposits on head and anterior part of body (Figure 4 C); its upper eyelid with a short, blunt conical horn *vs.* with a larger, rather long and pointed soft horn in the adult in latter (Figure 4 C). From *B. chuannanensis* by its occipital region significantly uplifted in males *vs.* not uplifted; having a distinct transverse groove defines the head behind in males *vs.* absent same groove in latter.

3.4 Remarks All specimens of *B. popei* sp. nov. were found ensconced among rocks in montane streams surrounded by moist subtropical evergreen broadleaved forests at elevation between 900 m–1300 m a.s.l. (Figure 5). During July to September, males emit a series of short loud notes repeated 12–17 times with mean 0.410 s of inter-note intervals from hidden positions. All male specimens collected during July to August, presenting dense tiny black nuptial spines on the dorsal surface of the first finger and second finger. The male paratype SYS a000584, collected on 13 August, was bearing mature testes in the abdominal cavity, measuring 7.7 mm × 4.8 mm × 2.8 mm. The female paratype SYS a001875, collected on 20 July, was gravid, with pure yellowish oocytes in the oviduct; oocytes spherical, measuring 2.9 mm–3.5 mm. Tadpoles at Gosner stages 26th–29th were found under rocks in the stream on 7 April and 6 December 2011. Thus, the breeding season is from July to September.

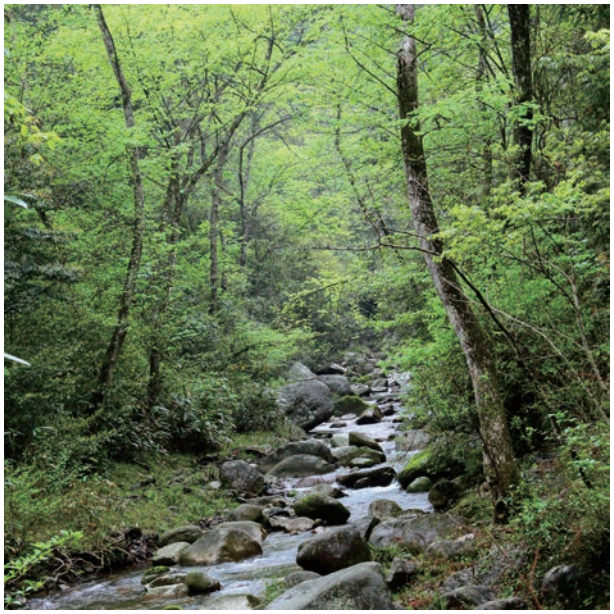

Figure 5 Habitat of *B. popei* sp. nov. at the type locality Mount Jinggang, Jiangxi Province. Photo by Jian ZHAO on 11 April 2011.

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Appendix 1: Specimens Examined

Brachytarsophrys chuannanensis (n = 1): CIB98A0045, Holotype, Zihuai, Hejiang County, Sichuan Province, China, 850 m a.s.l.

Brachytarsophrys carinense (n = 5): KIZ91002, 91002, 90267, 90273, adult males; KIZ910270, adult female, Santai, Dayao County, Yunnan Province, China, 2100–2700 m a.s.l.

Brachytarsophrys feae (n = 6): Two adult males, SYS a001770–1771, and one tadpole, Heping Town, Zhenyuan County, Yunnan Province, China, 12 May 2012; KIZ409177/2B-1-6, three tadpoles, Kungang Vallige, Jingdong County, Yunnan Province, China, 1850 m a.s.l., 21 August, 1984.